MACSima™ Imaging Cyclic Staining (MICS)
Sample preparation protocol for adherent cells

Miltenyi Biotec

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Material:

- Adherent cell line
- MACSwell™ Sealing Foils (Miltenyi Biotec, #130-126-866)
- MACSwell™ 24 Imaging Plates (Miltenyi Biotec, # 130-124-677)
- 1X PBS with calcium and magnesium, prepared from 10X PBS (e.g. Biowest, # X0520-500)
- MACSima Running Buffer (Miltenyi Biotec, # 130-121-565)
- Paraformaldehyde (PFA) solution 4 % (e.g. Biozol, # BOB-AR1068)
- DAPI Staining Solution (Miltenyi Biotec, component of MACSima Stain Support Kit: # 130-127-574 (human), # 130-127-575 (mouse))

Protocol

- Cultivate adherent cells on a MACSwell 24 Imaging Plate in medium recommended for the particular cell line. For determination of the proper cell density for the respective cell line, seed different cell numbers into different wells, so the wells with the optimal cell number can be chosen for the experiment.

  Note: MACSwell 24 Imaging Plate is not coated. If coating is needed, perform a coating step before cell seeding. Also, note that a maximum of four wells can be selected for imaging in the MACSima Imaging System.
- After cultivation, aspirate supernatant / medium gently.
- Wash cells carefully with 300 µL PBS by adding PBS gently to one corner of the well and aspirating it on the opposite corner.
- After last washing step / aspiration, fix cells with 300 µL 4% PFA per well for 10 min at room temperature.
- Aspirate supernatant / PFA gently.
- Wash cells three times with 300 µL PBS by adding PBS gently to one corner of the well and aspirating it on the opposite corner.
- Right before the start of the MICS experiment, perform a DAPI pre-staining. Prepare the DAPI pre-staining solution by diluting DAPI Staining Solution at 1:25 (0.2 µg/ml) in MACSima Running Buffer at final volumes of 300 µL per well of a MACSwell 24 Imaging Plate.

Note: The optimal dilution factors of DAPI Staining Solution may vary depending on cell type and might need to be adapted.

- Remove the PBS from the wells by pipetting.
- Add the DAPI pre-staining solution and incubate for 10 minutes in the dark at room temperature.
- Afterwards, remove the DAPI pre-staining solution and wash the wells three times with 300 µL MACSima Running Buffer per well of a MACSwell 24 Imaging Plate.

  Note: Add MACSima Running Buffer gently to one corner of the well and aspirate on the opposite corner; repeat this for all four corners.
- After last buffer aspiration, add 475 µL MACSima Running Buffer (initial sample volume) per cell-containing well of a MACSwell 24 Imaging Plate.

  Note: Make sure that the initial sample volume is correct before loading the sample into the MACSima Imaging System. If the plate is not immediately used for loading into the MACSima Imaging System, cover the plate with MACSwell Sealing Foil.
- Follow the instructions of the MACSima Imaging System Hardware User Manual & the instrument software to start the experiment.

  Note: Do not image at the edge of the well or near to the corner.